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PATENT APPLICATION SERIAL NO._____

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE
FEE RECORD SHEET

PTO-1556
(5/87)

BE IT KNOWN that

Christopher K. Ngichabe of Ithaca, New York; and

Fredric W. Scott of Brooktondale, New York

did invent certain new improvements in

RECOMBINANT VIRAL VACCINE

the following disclosure of which contains a correct and full description of the invention and the best mode known to the inventors for taking advantage of the same.

RECOMBINANT VIRAL VACCINE

Abstract Of The Disclosure

The present invention describes an infectious recombinant of raccoon poxvirus with an exogenous DNA element selected from a second infectious viral genome which is capable of expressing the protein coded for by the DNA element in an infected mammal and thereby serves as a vaccine virus providing a means for bringing about antibody formation in the infected mammal to the second infectious viral genome.

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501 RECOMBINANT VIRAL VACCINE

Feline panleukopenia is considered the most devastating disease of cats in unvaccinated populations. Although vaccines, both modified live virus and inactivated virus, are available and currently the focal point of all immunizations programs in the cat, more efficacious vaccines are still needed. The present invention is directed to such efficacious vaccines.

In addition, there are a number of diseases for which effective vaccines are not available. As recombinant viral vaccines are developed against these diseases, multivalent vaccines based upon the recombinant construct according to the present invention can be developed with relative ease to provide for a number of multivalent vaccines.

As there are estimated to be over 50,000,000 cats in the United States and an equal number overseas, and as every cat must be vaccinated to protect it against FPV if it is to remain healthy, the importance of the invention described herein is readily apparent.

Feline panleukopenia is a highly contagious viral disease of domestic and exotic cats characterized by sudden onset, fever, anorexia, depression, leukopenia, vomiting and diarrhea, dehydration, and often a high mortality rate. In unvaccinated populations, feline panleukopenia is the most devastating disease of cats known. The causative agent of feline panleukopenia is a virus, feline parvovirus, and the disease is known under a number

of different names such as feline parvovirus, feline panleukopenia, infectious enteritis, viral enteritis, gastroenteritis, cat "distemper", granulocytosis, feline ataxia, cat plague, cat fever, and show plague.

The disease occurs worldwide and was most probably the cause of the great cat plagues of ancient times. Because of the extreme resistance of the virus to environmental conditions, contaminated premisses remain a source of infection for years after a clinical case occurs on that premises.

The incidence of clinical feline parvovirus varies, depending on the percentage of immune cats in the population, the virulence of the particular strain of virus, and the virulence of intestinal bacteria in the infected cats. Virtually 100% of susceptible cats on a premise that come in contact with an infected cat or contaminated environment become infected with virus; some develop a subclinical infection, others a mild infection, while others develop a serious disease.

The mortality from FP varies from 25% to as high as 90% and usually averages about 50%.

There tends to be a seasonal incidence for feline parvovirus coinciding with the buildup of a susceptible population as young kittens lose their protective maternally-derived immunity. The exact time of the year when this higher incidence occur depends on the seasonal breeding for that locality. However, feline parvovirus can and does occur at any time of the year.

While feline panleukopenia is predominantly a disease of young kittens 2 to 4 months of age, it can affect cats of all ages. Older cats, however, are more likely to develop subclinical or mild infections compared to the more severe disease in kittens. There is no sex predilection for this disease.

The domestic cat is the primary host of feline parvovirus, but all members of the Family Felidae are believed to be susceptible to feline parvovirus including tigers, leopards, wild cats, lynxes, servals, leopard cats, tiger cats, ocelots, cheetahs, lions, snow leopards, and panthers

In addition, members of the *mustilidae* such as mink are highly susceptible to the mink strain of virus (mink enteritis virus, MEV), but have subclinical infections with the cat strain of feline parvovirus. Ferrets can be infected *in utero* or neonatally. Little information is available concerning the susceptibility of other *mustilidae* such as skunks, otters, weasels, and badgers to the virus. In addition, members of the *procyonidae* (raccoons and coati-mundi) are also highly susceptible to feline parvovirus.

Until a worldwide pandemic of canine parvovirus in 1977 to 1978, *canidae* were generally believed to be resistant to feline parvovirus. Infection of dogs with the feline virus is an abortive infection with limited replication and no shed of the feline virus. The origin of the canine strain of parvovirus is not known, but it is generally assumed to be a mutant virus from cats or wildlife.

Transmission of the virus is usually by direct contact of susceptible cats with infected cats, since virus is excreted

during the acute phase of the illness in all body secretions and excretions. Contaminated feed and water dishes, cages, bedding, litter boxes, rugs, and soil can serve as sources of the virus for many months and perhaps years. Virus can be transmitted on contaminated clothing, shoes, and hands of people. The rapid transmission of the related canine parvovirus throughout the world probably occurred in this way.

Aerosol transmission may occur, especially if the cat is co-infected with respiratory viruses so that sneezing occurs. Insects and parasite, especially fleas, can transmit the virus as mechanical vectors.

The pathogenesis of feline parvovirus infection depends on the state of mitotic activity of the various tissues within the body. Virus enters the cat via the oral route and primary infection occurs in the lymphoid tissues of the oral pharynx. The regional lymph nodes then become infected. Within 24 hours after ingested virus, the cat is viremic, and the virus is distributed throughout the body. The epithelial crypt of the ileum and jejunum of the small intestine are particularly susceptible to the virus. Cytolytic replication of the virus in these cells destroys the epithelial lining of the crypts which results in ballooned, debris-filled crypts and shortened, blunted villi. If the mitotic rate of the crypt cells is low, such as occurs in germ free kittens, the virus only destroys an occasional crypt cell and does not produce gross or microscopic lesions of the intestine. Other

tissues with rapidly dividing cells (thymus, bone marrow, lymph nodes) are affected by the cytolytic replication of the virus.

Viremia lasts for 7 to 8 days after exposure (approximately the third or fourth day of illness), at which time virus-neutralizing antibodies appear in blood. The antibody titer increases rapidly and reaches its maximum by about 14 days after exposure. With the appearance of antibody, virus in most tissues gradually disappears. However, a small amount of virus may persist intracellularly where it is protected from antibodies for periods of several weeks, months, or even years in certain tissues such as kidney. Shed of infectious virus is not a common finding in cats recovered from the disease. Generally by 3 weeks after infection, cats no longer shed infectious virus in the feces, urine, or other secretions or excretions. Virus that may be shed from chronically infected tissues is quickly neutralized by antibody present in these tissues or excretions. However, virus can be isolated from feces of a small percentage of recovered cats for several weeks.

If infection with feline parvovirus occurs in a pregnant cat, the virus readily infects the uterus and crosses the placenta to infect the fetus. Infection spread throughout the fetus and crosses the blood brain barrier to infect the cerebellum and other tissues within the central nervous system. The result of this infection depend on the stage of gestation at the time of infection. The possibilities include abortion, stillbirths, early neonatal deaths, or teratological changes, especially cerebellar

hypoplasia. Evidence suggests that hydrocephalus and hydroencephaly may be a result of feline parvovirus infection *in utero*.

In utero or neonatal infection can result in cell damage in the retina of the eye, leading to retinal dysplasia, but without loss of visual acuity, focal retinal lesions in 31% of kittens with naturally occurring cerebellar degeneration and ataxia due to feline parvovirus, compared to less than 2% in the general feline population have been found.

Infection of queens during the first half of gestation can result in death of one or more fetuses with total resorption of dead fetuses and placental membranes if abortion does not occur. Later in gestation, resorption of fluids from the dead fetus and placenta results in a dehydrated mummified fetus which may be retained until term. Susceptible queens infected during the latter half of gestation may give birth to litters containing normal healthy kittens, stillborn kittens, partially autolyzed fetuses and mummified fetuses.

Infection of uterus and fetuses only occurs when completely susceptible queens undergo an acute infection. Queens that have undergone a previous infection will have neutralizing antibodies which will protect the fetuses. Similarly, subsequent litters to a feline parvovirus-affected litter will not experience problems from the virus.

The immune response to feline parvovirus infection is rapid and solid. Cats that have undergone natural infection are immune

for life, and as stated discussed above, there are numerous commercial vaccines available to immunize cats against the virus.

The clinical signs of feline panleukopenia have been described by many authors. The incubation period for the disease can vary from 2 to 7 days, but is normally about 4 to 5 days. Clinical illness usually has an acute onset. The severity of illness of cats infected with feline parvovirus from a completely subclinical or asymptomatic infection to one which is rapidly fatal, resembling acute poisoning.

In the typical case of feline panleukopenia, there is a sudden onset of clinical signs. The cat may have a temperature of 40°C (104°F) or higher and show depression and complete anorexia. Vomiting usually occurs and a severe fetid diarrhea may develop within 24 to 48 hours. Blood and casts may be passed in the feces. If vomiting and diarrhea continues, severe dehydration and electrolyte imbalances usually occur.

Cats with feline panleukopenia often assume a typical attitude or posture, hunches up with their head between their paws. they frequently will hang their heads over a water dish or feed dish. acting as if they would like to drink and may even take a lap or two of milk or water, but they are unable or are reluctant to swallow. Their hair coat becomes rough and dull, and there is a loss of elasticity of the skin due to the dehydration. The third eyelids often appear prominent. The abdomen is painful and abdominal palpation elicits signs of pain. The mesenteric nodes

are enlarged and the gastrointestinal tract contains excess gas and liquid.

Terminally, a subnormal temperature is observed, indicating a grave prognosis. Coma and death follow in a few hours. The mortality in the acute form of the disease may vary from 25 to 90%. Death may occur within the first 5 days of the illness in uncomplicated cases, or after 5 days in complicated cases. If the cat survives approximately 5 days of illness and secondary complications such as bacterial infections, severe dehydration, or chronic enteritis from concurrent infections do not occur, then recovery should be fairly rapid. It will take several weeks for the cat to regain its lost weight and condition.

Feline panleukopenia is both overdiagnosed and underdiagnosed by the general practice veterinarian. To diagnose feline panleukopenia for all seriously ill cats with a leukopenia is to overdiagnose the disease, but on the other hand, the disease may be misdiagnosed, especially in the peracute and mild forms.

The diagnosis is based on history, clinical signs, and the presence of leukopenia, and can be confirmed by gross and microscopic changes, and by various laboratory tests.

The history will often point to a diagnosis of feline panleukopenia, especially age, vaccination records, and contact with strange cats within the past 2 weeks (e.g., a boarding kennel, hospital, or adoption shelter). A sick, unvaccinated cat less than 1 year of age is highly suspect to feline panleukopenia. Moreover, a history of vaccination does not rule out the

possibility of the disease. Maternally-derived immunity can interfere with vaccination and leave a kitten susceptible to the disease after the maternally-derived immunity has waned. This is more likely to occur if the kitten was last vaccinated when less than 12 weeks of age.

An older cat vaccinated as a kitted without periodic revaccinations may lose its immunity after a few years. How common an occurrence this is remains unknown.

The most characteristic finding in feline panleukopenia is the leukopenia which occurs in almost all feline parvovirus-infected cats, even if they do not show clinical signs. There is usually a direct correlation between the severity of the leukopenia and the severity of the disease. This leukopenia is characterized by a progressive drop in circulating white blood cells 1 to 2 days prior to the development of clinical signs, with a precipitous drop on the day of the crisis. The leukocyte count is usually 4000 to 8000 in subclinical infections and less than 4000 in clinical infections. Counts below 2000 warrant a guarded prognosis. Due to the extreme reduction in neutrophils, a relative lymphocytosis may occur, but as the disease progresses the lymphocytes may disappear also. A count of 0 to 200 leukocytes per deciliter of blood is not unusual. If the cat survives for approximately 5 days after the onset of signs, there is a dramatic rebound in the total leukocyte count (with a marked left shift) often exceeding the upper normal limit in another 3 to 4 days.

The diagnosis of feline parvovirus can be confirmed by viral isolation, serological tests, or pathological changes. Viral isolation can be done in feline cell cultures or by immunofluorescence. Swabs may be taken from the pharynx or from the rectum and places in viral-transport medium, and then submitted to a diagnostic laboratory that is equipped to do feline viral isolations. The best tissue to submit for viral isolation from autopsied animals are spleen, thymus, ileum, or mesenteric lymph node. These samples should be placed in sterile vials and either transmitted directly to the laboratory or frozen and submitted under dry ice refrigeration. For immunofluorescence, tissues from autopsied animals should be snap frozen in liquid nitrogen and submitted for sectioning and staining. Impression smears can also be taken of the spleen or mesenteric lymph node, fixed in cold acetone, and the dried slide then submitted to the diagnostic laboratory. Electron microscope examination of fecal samples for typical parvovirus particles also will confirm the diagnosis.

For the serological diagnosis of FP, the viral neutralization (VN) test is the test of choice. The ELISA and hemagglutination inhibition test can also be used. One serum sample is taken during the acute phase of the disease and a second sample is taken 2 weeks later. The serum samples (1 ml each) should be frozen until submitted to the laboratory, but they can be shipped by regular mail without refrigeration. Paired serum samples are required since results of a single sample are meaningless in

establishing a diagnosis. Hemolysis does not affect the validity of the VN test.

The pathological changes in feline panleukopenia have been described by several individuals. Cats that die from the disease appear gaunt and dehydrated as evidenced by the sticky, dry tissues and the sunken, soft eyes. there is usually evidence of diarrhea and vomiting.

The gross pathological changes in feline panleukopenia may be relatively mild and not by the casual observer. Careful observation usually shows changes in the small intestine, primarily the ileum and jejunum. The intestine is usually dilated and edematous with a turgid, hoe-like appearance. Often there is a hyperemia and/or petechial hemorrhages on the serosal and mucosal surfaces. The feces are scant and watery and have a fetid odor and a yellowish gray appearance. The mesenteric lymph nodes are edematous and may be hemorrhagic.

In kittens suffering from feline ataxia due to infection with feline parvovirus, there is a gross reduction in the size of the cerebellum. Kittens with feline parvovirus induced hydrocephalus have an enlarged cranium with enlarged cerebral hemispheres due to dilated lateral and third ventricles. The cerebral cortex is thinned from increased pressure of the cerebrospinal fluid.

Newborn kittens that die from feline panleukopenia usually have minimal gross pathological changes. The main lesion is a degeneration of the thymus. Hemorrhagic encephalopathy occasionally may be observed.

The histopathological changes are primarily restricted to those tissues which are undergoing active cell mitosis. The most consistent and striking lesions are in the epithelium of the crypts of the small intestine, especially the ileum and jejunum. These crypts are ballooned and filled with debris. The epithelial cells lining the crypts are undergoing degeneration or may be sloughed off entirely. The villi of the intestine are shortened due to sloughing of the tips of the villi. The bone marrow and the lymphoid tissues such as the mesentric lymph modes, spleen, and thymus have a marked reduction in cellular elements. Vessels in all organs usually are devoid of leukocytes. The liver often has a dissociation of hepatic cells. Intranuclear inclusions may be observed, especially in the crypt epithelial cells of the small intestine early in the disease.

Disseminated intravascular coagulation has been reported in cases of feline panleukopenia in domestic cats and *F. sylvestris*. Numerous microthrombi may occur in peripheral blood vessels in renal medulla and cortex, liver, heart, lungs, and occasionally in other organs.

In the feline parvovirus induced cerebellar hypoplasia, there is a decrease or absence of the granular cells of the cerebellum. The Purkinje cells are decreased in number and scattered. There is no correlation between the degree of hypoplasia and the clinical signs exhibited.

Tissues of newborn kittens with feline panleukopenia have widespread intranuclear inclusions, especially in the heart.

The animals susceptible to feline parvovirus may be vaccinated with a vaccine prepared according to the following invention which relies upon the formation of a construct comprising a genomic element from a virus to which protection is desired in combination with a carrier virus genome. Although a number of carrier virus genomes may be used in accordance with the present invention such as vaccinia, fowl pox, and herpes (feline herpes virus) virus, the preferred carrier virus is raccoon poxvirus.

Infectious raccoon poxvirus recombinants expressing the parvovirus capsid proteins (VP2) gene are specifically described herein as merely one example of the utility of the present invention. As will become apparent to the reader, however, the present invention should not be considered to be so limited in scope so as to only be relevant to feline parvovirus.

Raccoon poxvirus was first isolated from the upper respiratory tract of healthy raccoons in Maryland [see Bacteriol. Proc. 64th Annual Meeting, Amer. Soc. Microbiol., pg 117 (1964)]. The virus which was found in 22 of 92 animals tested, produced a hemagglutinating antigen which was inhibited by an antiserum to vaccinia virus. Subsequent reports have identified the virus as being closely related to vaccinia and cowpox, but sufficiently different to be considered a new member of the vaccinia/variola subgroup of poxviruses. Recent hybridization experiments have shown that the virus contains the same thymidine kinase (Tk) gene nucleotide sequence as vaccinia, thus suggesting that

chimeric plasmids designed for inserting heterologous coding sequences into the vaccinia virus Tk region might be suitable for inserting such DNA sequences into the genome of the raccoon poxvirus.

Recently, a live raccoon poxvirus recombinant for expressing rabies virus surface glycoprotein has been successfully used for oral rabies vaccination of raccoons against rabies [see Virology 165:313 (1988)]. Cats have also been successfully immunized against rabies by this recombinant. We have screened several poxviruses for cat immunization potential, and have shown concluded that raccoon poxvirus was both safe and highly immunogenic. Given the controversy over the use of vaccinia virus recombinant vaccines due to the rare side effects in both animals and humans, and its inability to provoke sufficiently high serum neutralizing antibodies, raccoon poxvirus as a carrier virus stands out to be the best choice for feline poxvirus recombinant vaccine. One such raccoon poxvirus which has been found to be acceptable for the purposes of the present inventon is that designated as American Type Culture Collection Accession No. VR2212.

Accordingly, it is one aspect of the present invention to describe the successful insertion of an exogenous gene from one viral genome into a chimeric plasmid, and the transfection of this plasmid into the thymidine kinase portion of raccoon poxvirus.

It is another aspect of the present invention to utilize the infectious raccoon poxvirus for expressing an exogenous gene from a second viral source and its use in the immunization of animals against the second virus.

More specifically, it is a major aspect of the present invention to describe the successful insertion of the gene that codes for the VP2 capsid protein of FPV into a chimeric plasmid, and the transfection of this plasmid into the thymidine kinase portion of raccoon poxvirus.

In addition, it is still another major aspect of the present invention to describe the development of an infectious raccoon poxvirus for expressing the feline parvovirus capsid protein (VP2) and its use in the immunization of cats against feline parvovirus.

These and other aspects of the present invention will become more apparent to the reader after consideration of the following figures, examples, and detailed description of the invention and the manner and process of using it. Although the following description emphasizes the use of raccoon poxvirus and feline parvovirus in making and characterizing the present invention, it is to be remembered that the following figures and examples are presented for solely for the purpose of more completely illustrating the present invention, and thus they are not intended to limit the scope of the present invention, nor should they be read as doing so, in any manner.

With reference to the figures:

FIGURE 1 depicts a subcloning flow-chart showing the strategy of constructing the chimeric plasmid construct according to the present invention from the feline parvovirus DNA;

FIGURE 2 depicts a flow-chart showing the strategy of constructing the recombinant virus according to the present invention from the chimeric plasmid depicted in Fig. 1;

FIGURE 3 is a photographic representation of the Restriction enzyme analysis of the chimeric transfer plasmid according to the present invention to determine the orientation of the parvovirus DNA;

FIGURE 4 is a photographic representation of the genomic analysis of the recombinant raccoon pox/feline parvovirus according to the present invention;

FIGURE 5 is a photographic representation of immunoblots of raccoon pox/feline parvovirus recombinant virus according to the present invention grown in either A72 or BSC40 cells and probed with 1:100 dilution of FVP polyclonal antiserum;

FIGURE 6A is a photographic representation of dot blot hybridization of positive recombinant virus according to the present invention after three plaque purifications in 143B cells; and

FIGURE 6B is a photographic representation of dot blot hybridization of plaques after three passages of transfection mixture in 143B cells and once in vero cells.

The plasmid PTKgptF3S carrying an insert of DNA which expresses the VP2 capsid protein of feline parvovirus, according to one preferred embodiment of the present invention, has been deposited and accepted under the provisions of the Budapest Treaty into the American Type Culture Collection, Rockville

Maryland under Accession Number _____. Upon request, the Commissioner of Patents and Trademarks shall have access to the deposit which shall be viably maintained for a 30 year period, or for 5 years following the last request, or the life of the patent, whichever is longer. Upon issuance of the patent, the plasmid shall be made available to the public in accordance with the law and with appropriate restrictions and guidelines applicable to the safe handling and use of this class of infectious viruses.

The genome of feline parvovirus has been cloned and sequenced [see Virol. 55:574 (1985)], with the virons containing generally three size classes of protein: a large 80-85 Kda VP1 protein which makes up to 10-15% of the viral protein; a smaller VP2' portion of 64-67 Kda which makes up about 85-90% of the viral protein; and a portion of the VP2' protein following proteolytic cleavage to yield a 60-64 Kda species (VP2). In making the present invention, the VP2-encoding genome was the one isolated and inserted into the chimeric plasmid to achieve the recombinant virus used to express the protein and provide antibody production in animals.

The amino acid sequence of the VP2 protein is

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Met Ser Asp Gly Ala Val Gln Pro Asp Gly Gly Gln Pro Ala Val
1               5                  10                  15
Arg Asn Glu Arg Ala Thr Gly Ser Gly Asn Gly Ser Gly Gly Gly
                20                  25                  30
Gly Gly Gly Gly Ser Gly Gly Val Gly Ile Ser Thr Gly Thr Phe
                35                  40                  45
Asn Asn Gln Thr Glu Phe Lys Phe Leu Glu Asn Gly Trp Val Glu
                50                  55                  60
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Ile Thr Ala Asn Ser Ser Arg Leu Val His Leu Asn Met Pro Glu
65 70 75
Ser Glu Asn Tyr Lys Arg Val Val Val Asn Asn Met Asp Lys Thr
80 85 90
Ala Val Lys Gly Asn Met Ala Leu Asp Asp Thr His Val Gln Ile
95 100 105
Val Thr Pro Trp Ser Leu Val Asp Ala Asn Ala Trp Gly Val Trp
110 115 120
Phe Asn Pro Gly Asp Trp Gln Leu Ile Val Asn Thr Met Ser Glu
125 130 135
Leu His Leu Val Ser Phe Glu Gln Glu Ile Phe Asn Val Val Leu
140 145 150
Lys Thr Val Ser Glu Ser Ala Thr Gln Pro Pro Thr Lys Val Tyr
155 160 165
Asn Asn Asp Leu Thr Ala Ser Leu Met Val Ala Leu Asp Ser Asn
170 175 180
Asn Thr Met Pro Phe Thr Pro Ala Ala Met Arg Ser Glu Thr Leu
185 190 195
Gly Phe Tyr Pro Trp Lys Pro Thr Ile Pro Thr Pro Trp Arg Tyr
200 205 210
Tyr Phe Gln Trp Asp Arg Thr Leu Ile Pro Ser His Thr Gly Thr
215 220 225
Ser Gly Thr Pro Thr Asn Ile Tyr His Gly Thr Asp Pro Asp Asp
230 235 240
Val Gln Phe Tyr Thr Ile Glu Asn Ser Val Pro Val His Leu Leu
245 250 255
Arg Thr Gly Asp Glu Phe Ala Thr Gly Thr Phe Phe Phe Asp Cys
260 265 270
Lys Pro Cys Arg Leu Thr His Thr Trp Gln Thr Asn Arg Ala Leu
275 280 285
Gly Leu Pro Pro Phe Leu Asn Ser Leu Pro Gln Ser Glu Gly Ala
290 295 300
Thr Asn Phe Gly Asp Ile Gly Val Gln Gln Asp Lys Arg Arg Gly
305 310 315
Val Thr Gln Met Gly Asn Thr Asp Tyr Ile Thr Glu Ala Thr Ile
320 325 330
Met Arg Pro Ala Glu Val Gly Tyr Ser Ala Pro Tyr Tyr Ser Phe
335 340 345
Glu Ala Ser Thr Gln Gly Pro Phe Lys Ile Pro Ile Ala Ala Gly
350 355 360

Arg Gly Gly Ala Gln Thr Asp Glu Asn Gln Ala Ala Asp Gly Asp
365 370 375
Pro Arg Tyr Ala Phe Gly Arg Gln His Gly Gln Lys Thr Thr Thr
380 385 390
Thr Gly Glu Thr Pro Glu Arg Phe Thr Tyr Ile Ala His Gln Asp
395 400 405
Thr Gly Arg Tyr Pro Ala Gly Asp Trp Ile Gln Asn Ile Asn Phe
410 415 420
Asn Leu Pro Val Thr Asn Asp Asn Val Leu Leu Pro Thr Asp Pro
425 430 435
Ile Gly Gly Lys Thr Gly Ile Asn Tyr Thr Asn Ile Phe Asn Thr
440 445 450
Tyr Gly Pro Leu Thr Ala Leu Asn Asn Val Pro Pro Val Tyr Pro
455 460 465
Asn Gly Gln Ile Trp Asp Lys Glu Phe Asp Thr Asp Leu Lys Pro
470 475 480
Arg Leu His Val Asn Ala Pro Phe Val Cys Gln Asn Asn Cys Pro
485 490 495
Gly Gln Leu Phe Val Lys Val Ala Pro Asn Leu Thr Asn Glu Tyr
500 505 510
Asp Pro Asp Ala Ser Ala Asn Met Ser Arg Ile Val Thr Tyr Ser
515 520 525
Asp Phe Trp Trp Lys Gly Lys Leu Val Phe Lys Ala Lys Leu Arg
530 535 540
Ala Ser His Thr Trp Asn Pro Ile Gln Gln Met Ser Ile Asn Val
545 550 555
Asp Asn Gln Phe Asn Tyr Leu Pro Asn Asn Ile Gly Ala Met Lys
560 565 570
Ile Val Tyr Glu Lys Ser Gln Leu Ala Pro Arg Lys Leu Tyr
575 580

A unique feature of the present invention is that the protein ultimately providing for the protection against feline parvovirus according to the present invention is non-glycoslylated and thus the ultimate antibody generation is contrary to accepted belief. The corresponding DNA codons for this capsid protein is:

1 ATG AGT GAT GGA GCA GTT CAA CCA GAC GGT GGT CAA CCT
40 GCT GTC AGA AAT GAA AGA GCT ACA GGA TCT GGG AAC GGG

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      79   TCT GGA GGC GGG GGT GGT GGT GGT TCT GGG GGT GTG GGG
     118   ATT TCT ACG GGT ACT TTC AAT AAT CAG ACG GAA TTT AAA
     157   TTT TTG GAA AAC GGA TGG GTG GAA ATC ACA GCA AAC TCA
     196   AGC AGA CTT GTA CAT TTA AAT ATG CCA GAA AGT GAA AAT
 5   235   TAT AAA AGA GTA GTT GTA AAT AAT ATG GAT AAA ACT GCA
     274   GTT AAA GGA AAC ATG GCT TTA GAT GAC ACT CAT GTA CAA
     313   ATT GTA ACA CCT TGG TCA TTG GTT GAT GCA AAT GCT TGG
     352   GGA GTT TGG TTT AAT CCA GGA GAT TGG CAA CTA ATT GTT
     391   AAT ACT ATG AGT GAG TTG CAT TTA GTT AGT TTT GAA CAA
10   430   GAA ATT TTT AAT GTT GTT TTA AAG ACT GTT TCA GAA TCT
     469   GCT ACT CAG CCA CCA ACT AAA GTT TAT AAT AAT GAT TTA
     508   ACT GCA TCA TTG ATG GTT GCA TTA GAT AGT AAT AAT ACT
     547   ATG CCA TTT ACT CCA GCA GCT ATG AGA TCT GAG ACA TTG
     586   GGT TTT TAT CCA TGG AAA CCA ACC ATA CCA ACT CCA TGG
15   625   AGA TAT TAT TTT CAA TGG GAT AGA ACA TTA ATA CCA TCT
     664   CAT ACT GGA ACT AGT GGC ACA CCA ACA AAT ATA TAT CAT
     703   GGT ACA GAT CCA GAT GAT GTT CAA TTT TAT ACT ATT GAA
     742   AAT TCT GTG CCA GTA CAC TTA CTA AGA ACA GGT GAT GAA
     781   TTT GCT ACA GGA ACA TTT TTT TTT GAT TGT AAA CCA TGT
20   820   AGA CTA ACA CAT ACA TGG CAA ACA AAC AGA GCA TTG GGC
     859   TTA CCA CCA TTT CTA AAT TCT TTG CCT CAA TCT GAA GGA
     898   GCT ACT AAC TTT GGT GAT ATA GGA GTT CAA CAA GAT AAA
     937   AGA CGT GGT GTA ACT CAA ATG GGA AAT ACA GAC TAT ATT
     976   ACT GAA GCT ACT ATT ATG AGA CCA GCT GAG GTT GGT TAT
25  1015   AGT GCA CCA TAT TAT TCT TTT GAA GCG TCT ACA CAA GGG
    1054   CCA TTT AAA ATA CCT ATT GCA GCA GGA CGG GGG GGA GCG
    1093   CAA ACA GAT GAA AAT CAA GCA GCA GAT GGT GAT CCA AGA
    1132   TAT GCA TTT GGT AGA CAA CAT GGT CAA AAA ACT ACT ACA
    1171   ACA GGA GAA ACA CCT GAG AGA TTT ACA TAT ATA GCA CAT
30  1210   CAA GAT ACA GGA AGA TAT CCA GCA GGA GAT TGG ATT CAA
    1249   AAT ATT AAC TTT AAC CTT CCT GTA ACA AAT GAT AAT GTA
    1288   TTG CTA CCA ACA GAT CCA ATT GGA GGT AAA ACA GGA ATC
    1327   AAC TAT ACT AAT ATA TTT AAT ACT TAT GGT CCT TTA ACT
    1366   GCA TTA AAT AAT GTA CCA CCA GTT TAT CCA AAT GGT CAA
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1405 ATT TGG GAT AAA GAA TTT GAT ACT GAC TTA AAA CCA AGA
1444 CTT CAT GTA AAT GCA CCA TTT GTT TGT CAA AAT AAT TGT
1483 CCT GGT CAA TTA TTT GTA AAA GTT GCG CCT AAT TTA ACA
1522 AAT GAA TAT GAT CCT GAT GCA TCT GCT AAT ATG TCA AGA
1561 ATT GTA ACT TAC TCA GAT TTT TGG TGG AAA GGT AAA TTA
1600 GTA TTT AAA GCT AAA CTA AGA GCA TCT CAT ACT TGG AAT
1639 CCA ATT CAA CAA ATG AGT ATT AAT GTA GAT AAC CAA TTT
1678 AAC TAT CTA CCA AAT AAT ATT GGA GCT ATG AAA ATT GTA
1717 TAT GAA AAA TCT CAA CTA GCA CCT AGA AAA TTA TAT

In addition to the specific sequence for the protein given above, the sequence continues as:

1753 TAATATACTT ACTATGTTTT TATGGTTATT ACATATCAAC TAGCACCTAG
2003 AAAATTATAT TAATATACTT ACTATGTTTT TATGTTTATT ACATATTATT
2053 TTAAGATTAA TTAAATTACA ACATAGAAAT ATTGTACTTG TATTTGATAT
2103 AGGATTTAGA AGGTTTGTTA TATGGTATAC AATAACTGTA AGAAATAGAA
2153 GAACATTTAG ATCATGGTTA GTATGGTATA CAATAACTGT AAGAAATAGA
2203 AGAACATTTA GATCATGGTT AGTAGTTTGT TTTATAAAAT GTAATTGTAA
2253 ACTATTAATG TATGTTGTTA TGGTGTGGGT GGTTGGTTGG TTTGCCCTTA
2303 GAATATGTTA AGGACCAAAA AAATCAATAA AAGACATTTA AAACTTAATG
2353 GTCTCGTATA CTGTCTATAA GGTGAACTAA CCTTACCATA AGTATCAACT
2403 TGTCTTTAAG GGGGGGGTGG GTGGGAGATG CACAATATCA GTAGACTGAC
2453 TG

Both of these sequences are available through IBI/Pustell Sequence Analysis Programs in GeneBank as "Restriction Analysis of PVFVP", the disclosure of which is herein incorporated in toto; both sequences begin at nucleotide 1,690 of the complete genome sequence of 3,942 nucleotides. However, in making the present invention, it was found that the nucleotide and peptide sequences beginning at the HincII restriction site (1722 of the GeneBank sequence or codon 12 or amino acid 12 in the above sequences),

were sufficient to provide expression of an immunogen from the inserted feline parvovirus gene since this was the location of the HincII restriction site shown cut in Figure 1.

The two Puc8 chimeric plasmids were obtained from the Baker Institute of Cornell University. One plasmid contained the upstream fragment of 1962 bp of feline parvovirus DNA sequence cloned in the ECOR1 and Pst1 sites, which the other had the downstream fragment of 1982 bp of parvovirus DNA cloned in the Pst1 and BamHI restriction endonuclease sites. The transfer chimeric plasmid, PTKgptF3s [see Virology 62:1849 (1988)] was obtained from the National Institutes of Health.

EXAMPLE I
(Construction of Chimeric Plasmid)

As depicted in Figure 1, 240 base pair Parvovirus DNA from the upstream fragment was cleaved from the puc8 chimeric plasmid by digestion with HincII and Pst1 restrictions enzymes under conditions recommended by the manufacturer (BRL Laboratories). The 1982 bp downstream fragment was cleaved using Pst1 and BamH1 restriction enzymes. The digests were electrophoresed in 1% agarose (Sigma Chemicals) in TBE buffer for 20 hours at 1.5V/cm. The gel was stained with ethidium bromide and the DNA fragments identified under UV transillumination. The fragments were then recovered by cutting out the gel where the DNA was located. The DNA was then purified from the gel by either electroelution or gean clean protocol. The HincII to PstI1 (240 bp) and the PSt1 to BamHI (1982 bp) parvovirus DNA were ligated into the transfer vector PTkgptF13S which had been cut with HincII and BamHI restriction enzymes following conventional procedures originally described by Maniatiss in 1982. The ligation mixture was used to transform DH5a *E. coli* cells according to the instructions of the supplier (BRL, Life Technologies, Inc. Gaithersburg). The transformed *E. coli* cells were plated on agar plates containing 100 µg/ml of ampicillin. The resultant colonies were screened for the presence of the chimeric plasmid by in situ colony hybridization [see PNAS 72:3961 (1975)]. Three colonies were identified and

designated as 5,51, 5.53, and 10.79. Correct orientation of the Parvovirus DNA in the transfer vector was confirmed by restriction enzyme mapping using ECoR1 which cut through the vector close to the HincII site and SPe1 which cut through the insert DNA at bp 2361. Two fragments of 0.639 Kb and 1.582 Kb, as predicted, were liberated. The chimeric plasmid 5.51 was further characterized by junction sequencing [see Virus Genes 1:7 (1987)] to establish that the insert was in frame and in the right Kozack context [see Cell Biology 108:229 (1989)]. For insertion into raccoon poxvirus, this plasmid was grown in bulk and purified by banding in cesium chloride gradients according to recognized protocols.

EXAMPLE II
(Insertion of Parvovirus DNA Into Raccoon Poxvirus)

Recombinant raccoon poxviruses were produced essentially as described by Mackett [see J. Virology 49:857] and depicted in Figure 2. Briefly, confluent 143 cells grown in the absence of bromodeoxyuridine in two 80 cm^2 tissue culture grade petri dishes were transfected with the biologically cloned wild type raccoon poxvirus at a multiplicity of infection (MOI) of 0.05 contained in 1.0 ml of innoculum using the calcium precipitation procedure. The plates were then incubated at 37°C for six hours when the medium was replaced with fresh medium, and incubation continued while monitoring the development of cytopathic effects (cpe). When the cpe was advanced, the cells were scraped off the plates using a rubber policeman, the medium containing the cell was harvested into 50 ml conical polypropylene tubes, and freeze-thawed through three cycles at -70°C. Recombinant virus was selected by plaque tituration in TK^- 143B cell monolayers grown in six well cluster plates under overlay medium containing 30 µg/ml bromodeoxyuridine. Individual virus plaques were grown in 143B cell cultures in 24 well plates, and screened for the presence of parvovirus VP2 gene by DNA dot blot hybridization [see DNA Cloning, vol 11, IRL Press, p 202 (1985)] using ^{32}P labelled (Amersham multiprime DNA labelling kit) parvovirus VP2 DNA as a probe. Recombinants were screened for expression by immune dot blotting. Recombinants were then plaque purified

three times in 143B cells in the presence of bromodeoxyuridine and once in CRFK cells without bromodeoxyuridine (see Figures 6A and 6B). Crfk monolayers were grown in T150 flasks supplemented by 10% fetal bovine serum, glutamine, nonessential amino acids, 0.1M NaOH, and penicillin/streptomycin. When 100% confluent, these cells were infected with the plaque purified recombinant virus at MOI of 0.05. When cpe was observed in 100% of the cells, they were harvested using a rubber policeman, and pelleted by centrifugation at 6000 rpm for 20 minutes in a GSA rotor. The cells were freeze-thawed through three cycles and sonicated (3 second bursts at 50W) and the virus was pelleted through a 40% sucrose cushion [see J. Virological Methods 2:175 (1981)]. The virus pellet was resuspended in TE Buffer (25 mM Tris-HCl pH 7.6) and stored at -70°C until used.

EXAMPLE III
(Analysis Of Recombinant Raccoon pox/Parvovirus Genome Structure)

Genomic DNA was isolated from raccoon pox and raccoon pox/parvovirus recombinant [see J. Virological Methods 2:175 (1981)]. Restricted DNA was electrophoresed in 0.65% agarose gel in TPE buffer at 0.7 V/cm for 24 hours. Southern blotting and hybridization were performed following conventional protocols.

EXAMPLE IV
(Analysis Of Protein Expression)

Parvovirus proteins were visualized by immunofluorescence, radioimmunoprecipitation (RIP), and western blotting of lysates of infected CRFK cells.

(a) Radioimmunoprecipitation: CRFK cell monolayers (25 cm^2) were infected with virus (30 pfu/cell). At 8 hours post infection, the monolayer was rinsed three times in methionine free media and 100 uci of ^{35}S labelled methionine (ICN) was added in 1.0 ml of methionine free medium (100 uci/ml). The flasks were then incubated at 37°C for two hours and harvested for radioimmunoprecipitation on 12% polyacrylamide gel.

(b) Western Blotting: Cell monolayers were infected at a multiplicity of infection as for RIP. At 18 hours post infection, lysates were prepared by washing the cells with cold PBS, scraping the washed cells off of the surface of the culture bottle, and pelleting them through a 40% sucrose cushion. The pelleted virus was then resuspended in distilled water and freeze-thawed through several cycles and stored at -70°C until used. For Western blotting, aliquots of pelleted cell lysates were electrophoresed in 12% (w/v) polyacrylamide gels [see J. Gen. Virol. 44:725 (1979)]. Polypeptides were transferred onto nitrocellulose at 60V for four hours in transfer buffer (50 mM Tris with 380 mM glycine, 20% methanol) using a transfer electrophoresis unit. The nitrocellulose filter was blocked with

3% bovine serum albumin (fraction V, Sigma Chemical Co.) in PBS and then incubated with feline parvovirus polyclonal antiserum (1:100 dilution) in PBS containing 0/05% Tween 20 for two hours at room temperature. The filter was washed several times with PBS/Tween 20 and incubated for 1 hour with cat antimouse horse radish peroxidase conjugated antibody. Polypeptide bands were visualized using 4-chloronaphthol in methanol and hydrogen peroxide dissolved in 10 mM Tris buffer at pH 7.4.

(c) Indirect immunofluorescence tests for parvovirus protein were performed with CRFK cells grown in 8 chamber Lab-Tek tissue culture slides and infected with recombinant virus at a multiplicity of infection of 30 pfu per cell. At 24 hours post infection, cell monolayers were rinsed twice with PBS and air dried with or without prior acetone fixation. Binding of virus specific antibodies was detected with fluorescein isothiocyanate conjugated cat antimouse IgG (Sigma).

EXAMPLE V
(Immunization Of Cats)

Two groups of 8 and 10 specific pathogen free cats and two in contact controls aged between 10 and 22 months were used. One group was vaccinated intranasally with 3.0×10^6 pfu of recombinant tissue culture virus in 1.0 ml of inoculum and boosted with the same amount of virus and the same route of administration 28 days post vaccination. At 132 days post vaccination, four cats were give 3.0×10^7 pfu of virus orally, and the other four were revaccinated subcutaneously at four sites with the same dose of virus. Blood samples were collected for serum on days 0, 14, 28, 47, 132, 146, and 160. The second group of ten cats were vaccinated with 2.8×10^9 pfu of purified recombinant virus using different routes of administration. Five cats were vaccinated subcutaneously at two sites, while the remaining five were vaccinated orally. At 21 days post vaccination, they were boosted with the same dose of virus in the same route. Blood samples were collected for serum on days 0, 14, 21 and 31.

EXAMPLE VI
(Detection of Antibody By Virus Neutralization Test)

To detect parvovirus neutralizing antibody, NLFK or CRFK cells were seeded in 8 chamber slides at 1 x 10^5 cells/ml, and incubated at 37°C for one hour. Similarly, two-fold serial dilutions of serum samples made in 96 well plates with 32 to 100 TCID 50/0.1 ml of feline parvovirus added were incubated at the same temperature and for the same duration. Following incubation, 0.1 ml of the serum/virus mixture was added to the cells and the slides were put in heat sealable bags and incubated at 37°C for 3-4 days. Virus neutralization titer was determined as end point serum dilution at which no parvovirus intranuclear inclusion bodies could be detected by the May Greenwald Giemsa stain. Raccoon poxvirus antibody was detected by the plaque reduction method. The results of these experiments are tabulated in the following Table 1:

TABLE 1

Serum Neutralizing Antibody Titers Against FPV and RPV of Cats Vaccinated Intranasally on Days 0 and 28 With 3 x 10^6 PFU of Recombinant FPV/RPV, and Revaccinated on Day 132

	Serum Neutralizing Antibody Titers (Days Post Vaccination)													
	FPV SN Titer							RPV SN Titer						
Cat No.	0	14	28	47	132*	147	160	0	14	28	47	132*	147	160
Vaccinates:														
U524	<16	22	>354	>2818	1000	2048	2048	<16	<16	<16	45	45	64	45
U363	<16	<16	<16	708	354	1000	1024	<16	<16	<16	22	16	45	89
U984	<16	<16	178	677	512	708	708	<16	<16	<16	32	22	64	45
U443	<16	<16	<16	133	64	1024	1024	<16	<16	<16	16	<16	45	45
U986	<16	<16	<16	<16	<16	512	708	<16	<16	<16	<16	<16	32	64
U121	<16	<16	<16	<16	<16	304	1000	<16	<16	<16	<16	<16	32	32
U533	<16	<16	<16	<16	<16	512	512	<16	<16	<16	<16	<16	45	64
U423	<16	<16	<16	304	128	1024	2048	<16	<16	<16	16	<16	64	89
Non-Vaccinated Controls:														
U353	<16	<16	<16	<16	<16	<16	<16	<16	<16	<16	<16	<16	<16	<16
U601	<16	<16	<16	<16	<16	<16	<16	<16	<16	<16	<16	<16	<16	<16

*Cats nos. U524, U363,U984,U443 were given a second booster vaccination (day 132) orally, while cats nos. U986, U121, U533, and U423 were vaccinated subcutaneously. Cats nos. U353, and U601 were the non-vaccinated contact controls.

The data in table 1 indicates the successful build-up of serum neutralizing antibody titers following vaccination with the recombinant virus according to the present invention. Although all animals responded with increased titers, some (those showing a titer of <16 on the 28th day) required a second vaccination with the recombinant virus. This second dosage was because the original dose (3×10^6 pfu) given was not sufficient to bring about antibody production in all animals. Subsequent testing indicated that a higher dose of approximately 3×10^8 pfu was sufficient to initiate antibody production in all animals. Furthermore, the production of antibody at this level was not dependent upon the route - intranasally, subcutaneous or oral - chosen for vaccination.

It can also be seen in this Table, and in those which follow, that in addition to the build-up of antibody to the parvovirus, there is also a slight build-up against the carrier virus, raccoon poxvirus. However, while it would be preferred in most instances that there would be no carrier virus antibody production, the amount produced in each instance can be considered to be relatively negligible.

EXAMPLE VII
(Detection of Antibody By Indirect Immunofluorescence Antibody Test)

CRFK cells were grown in 8 chamber Lab-Tech slides and infected with either feline parvovirus or raccoon poxvirus. Slides infected with feline parvovirus were incubated at 37°C for 3 to 4 days while those infected with raccoon poxvirus were incubated until cpe developed. The slides were then rinsed briefly in 0.1% bovine serum albumin (Fraction V) in PBS and then fixed in methanol and acetone. This was then followed by 3 to 6 rinses with 0.1% BSA in PBS and air drying. Serum samples diluted 1:16 to 1:64 (two wells per dilution) were applied and placed in a humid chamber for 1 hour at room temperature. Slides were then rinsed 3 to 5 times with 0.1% BSA in PBS and air dried. FITC-conjugated mouse anti cat antibody at 1:40 dilution was then added and the slides placed in a humid chamber at 37°C for 30 minutes. After rinsing with PBA/BSA, the slides were air dried and counterstained with a 1:500 dilution in PBS of 1% Evans blue stain for 10 to 20 minutes. The slides were then rinsed in plain PBS and air dried before mounting under coverslips and examined for fluorescence under a Zeis (model 7) microscope. The results, indicating the build-up of antibody against feline parvovirus in each instance, are contained in the following Table 2:

TABLE 2

Antibody Against FPV and RPV As Detected By Indirect Immunofluorescence Assay at 1:16 Dilution of Serum Samples For Cats Listed on Table 1

Cat No.	Antiviral Antibodies (Days post vaccination)					
	Feline Parvovirus			Raccoon Poxvirus		
	132	147	160	132	147	160
Vaccinates:						
U524	+++	++++	++++	++	++	++
U363	+++	++++	++++	++	++	++
U984	+++	++++	++++	+	++	++
U443	+++	++++	++++	-	++	++
U986	-	++++	++++	-	++	++
U121	-	++++	++++	-	++	++
U533	-	++++	++++	-	++	++
U423	+++	++++	++++	-	++	++
Non-Vaccinated Controls:						
U353	-	-	-	-	-	-
U601	-	-	-	-	-	-

EXAMPLE VIII

(Detection of Antibody By Hemagglutination Inhibition Test)

Hemagglutination inhibition titers were determined against canine parvovirus as described by Parrish et al [see Virology 166:293 (1988c)]. The results which are tabulated in the following Table 3, indicate that there is cross-reactivity between antibodies for feline parvovirus and canine parvovirus. Thus, the recombinant virus containing the construct of raccoon pox and feline parvovirus also has a potential for use as a vaccine to protect dogs from canine parvovirus infection.

TABLE 3

Hemagglutination Inhibition Antibody Titers Against Canine Parvovirus In Cats Listed in Table 1

Cat No.	HI Antibody Titer (Days post vaccination) 132	147	160
Vaccinates:			
U524	112	14125	7079
U363	20	14125	14125
U984	112	14125	7079
U443	112	7079	7079
U986	112	56234	56234
U121	112	3548	14125
U533	20	56134	56134
U423	112	56134	56134
Non-Vaccinated Controls:			
U353	<20	<20	<20
U601	<20	<20	<20

In addition to the above tables, the following Table 4 is presented to depict that in addition to the intranasal route of vaccine administration describe above and depicted in Figure 1, other routes of administration such as subcutaneous and oral vaccination with the recombinate virus according to the present invention is also successful in increasing antibody titer. In addition, Table 5 also confirms that the data in Table 3 is correct and that the recombinant virus according to the present invention is causing the vaccinated cats to produce an antibody which will react against canine parvovirus.

TABLE 4

Serum Neutralizing Antibody Titer Against FPV and RPV of Cats Vaccinated on Days 0 and 21 By Subcutaneous or Oral Route with 2.8 x 10^9 pfu of Purified RPV/FPV

Cat No.	Route of Vaccination	Serum neutralizing antibody titer (Days post vaccination)							
		FPV SN Titer				RPV SN Titer			
		0	14	21	31	0	14	21	31
Vaccinates:									
JD3	sc	<16	128	708	1995	<16	22	45	64
U985	sc	<16	354	708	1000	<16	45	89	89
U972	sc	<16	45	177	708	<16	22	64	128
U593	sc	<16	128	708	1024	<16	16	32	89
U516	oral	<16	89	177	354	<16	22	32	45
U514	oral	<16	64	354	354	<16	32	45	89
U513	oral	<16	89	256	708	<16	16	45	45
U591	oral	<16	45	128	708	<16	32	45	64
Non-Vaccinated Controls:									
U353	-	<16	<16	nt	<16	<16	<16	nt	<16
U601	-	<16	<16	nt	<16	<16	<16	nt	<16

sc = subcutaneous vaccination
FPV = feline parvovirus
RPV = raccoonpox virus

TABLE 5

Hemagglutination Inhibition Antibody Titers Against Canine Parvovirus In Cats Listed in Table 1

Cat No.	Route of Vaccination	HI antibody titers (days post vaccination)			
		0	14	21	31
JD3	sc	<20	1778	7079	7079
U985	sc	<20	3548	7079	7079
U972	sc	<20	3548	7079	56134
U593	sc	<20	1778	7079	7079
U516	oral	<20	20	112	223
U514	oral	<20	56	3548	14125
U513	oral	<20	<20	891	7079
U591	oral	<20	56	891	891

TABLE 5

Hemagglutination Inhibition Antibody Titers Against Canine Parvovirus In Cats Listed in Table 1

at o.	Route of Vaccination	[illegible] (days post vaccination) 0	14	21	31
D3	sc	<20	1778	7079	7079
1985	sc	<20	3548	7079	7079
1972	sc	<20	3548	7079	56134
1593	sc	<20	1778	7079	7079
1516	oral	<20	20	112	223
1514	oral	<20	56	3548	14125
1513	oral	<20	<20	891	7079
1591	oral	<20	56	891	891

As indicated earlier, the present invention may be modified by those skilled in the art to fit the needs of the particular vaccine sought. For example, the carrier virus need not be the infectious raccoon poxvirus, but may be other viruses such as fowl poxvirus, vaccinia virus, or herpes (especially feline herpes for vaccines directed to cats) virus.

In addition to a selected portion of the DNA from feline parvovirus as the "immunogen-producing" element of the recombinant construct, other immunogen-producing elements from other selected viruses such as, for example, feline calici virus, feline infectious peritonitis virus, bovine parvovirus and canine parvovirus, may be selected as the insert.

Multivalent recombinant vaccines are also within the scope of the present invention. For example, in addition to the feline parvovirus immunogen-producing DNA carried within the recombinant construct, additional immunogen-producing DNA elements from other disease causing viruses may be incorporated. For example, the raccoon poxvirus carrier genome may include 25 Kb of foreign DNA (the parvovirus insert is only approximately 2.5 Kb in length); it is therefore possible to provide additional DNA inserts from other disease causing viruses as, for example, hepatitis and/or herpes, and thus achieve protection to a number of different diseases with a single recombinant viral inoculation. Of course, with a RNA virus such as herpes, it would be necessary for the insert to be modified to correspond to the appropriate cDNA prior to insertion into the raccoon pox carrier virus

Simply put, if the recombinant carrier virus having an immunogen-producing nucleic acid insert in the viral genome, as for example the recombinant raccoon virus described in detail above, will replicate sufficiently in the host and to produce the immunogen that would protect against the virus from which the nucleic acid insert was selected, the recombinant virus could be used in the host animal to protect it against the disease caused by the virus from which the nucleic acid insert was taken.

In the figures which comprise a portion of the description of the present invention, Figures 1 and 2 are flow charts which are readily understood by those skilled in the art. Figure 3 depicts the analysis conducted to determine the size and orientation of the parvovirus DNA. In Figure 3, lanes 1 and 6 are known markers providing the size standard, lanes 2 and 7 is the wild-type parvovirus, and lanes 3-5 correspond to lanes 8-9 and indicate that the chimeric plasmid contains the DNA insert in the proper orientation. Figure 4 indicates that the DNA insert has been inserted in the correct location; the upper two depictions correspond to the lower two depictions with the exception that the lower two depictions have been over-exposed to increase sensitivity. In Figure 4, lanes 1 and 8 are identical and represent λHindIII markers for size determination; lanes 2, 5, 9 and 12 are identical and represent the chimeric plasmid cut with ECOR1 and BamHI; lanes 3, 6, 10 and 13 are identical and represent the wild type raccoon poxvirus; lanes 4, 7, 11 and 14 are identical and represent the recombinant type (raccoon poxvirus/feline

parvovirus) cut with HindIII. Figure 5 indicates that the preparation is pure and that the recombinant is expressing the gene to provide reaction with feline parvovirus polyclonal antiserum.

Thus, while we have illustrated and described the preferred embodiment of our invention, it is to be understood that this invention is capable of variation and modification, and we therefore do not wish to be limited to the precise terms set forth, but desire to avail ourselves of such changes and alterations which may be made for adapting the invention to various usages and conditions. Accordingly, such changes and alterations are properly intended to be within the full range of equivalents, and therefore within the purview of the following claims.

Having thus described our invention and the manner and a process of making and using it in such full, clear, concise and exact terms so as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same;

WE Claim:

1. A plasmid designated as PTKgptF3S and having an exogenous nucleic acid insert selected from an immunogen-producing portion of the genome of a disease causing virus of animals.

2. A plasmid according to Claim 1 wherein the nucleic acid insert is selected from the nucleic acid contained within a virus which codes for a viral capsid protein.

3. A plasmid according to Claim 2 wherein the protein is feline parvovirus capsid protein PV2.

4. A plasmid according to Claim 2 in which the nucleic acid insert comprises the sequence:

1 ATG AGT GAT GGA GCA GTT CAA CCA GAC GGT GGT CAA CCT
40 GCT GTC AGA AAT GAA AGA GCT ACA GGA TCT GGG AAC GGG
79 TCT GGA GGC GGG GGT GGT GGT GGT TCT GGG GGT GTG GGG
118 ATT TCT ACG GGT ACT TTC AAT AAT CAG ACG GAA TTT AAA
157 TTT TTG GAA AAC GGA TGG GTG GAA ATC ACA GCA AAC TCA
196 AGC AGA CTT GTA CAT TTA AAT ATG CCA GAA AGT GAA AAT
235 TAT AAA AGA GTA GTT GTA AAT AAT ATG GAT AAA ACT GCA
274 GTT AAA GGA AAC ATG GCT TTA GAT GAC ACT CAT GTA CAA
313 ATT GTA ACA CCT TGG TCA TTG GTT GAT GCA AAT GCT TGG
352 GGA GTT TGG TTT AAT CCA GGA GAT TGG CAA CTA ATT GTT
391 AAT ACT ATG AGT GAG TTG CAT TTA GTT AGT TTT GAA CAA
430 GAA ATT TTT AAT GTT GTT TTA AAG ACT GTT TCA GAA TCT
469 GCT ACT CAG CCA CCA ACT AAA GTT TAT AAT AAT GAT TTA
508 ACT GCA TCA TTG ATG GTT GCA TTA GAT AGT AAT AAT ACT
547 ATG CCA TTT ACT CCA GCA GCT ATG AGA TCT GAG ACA TTG
586 GGT TTT TAT CCA TGG AAA CCA ACC ATA CCA ACT CCA TGG
625 AGA TAT TAT TTT CAA TGG GAT AGA ACA TTA ATA CCA TCT
664 CAT ACT GGA ACT AGT GGC ACA CCA ACA AAT ATA TAT CAT

703 GGT ACA GAT CCA GAT GAT GTT CAA TTT TAT ACT ATT GAA
742 AAT TCT GTG CCA GTA CAC TTA CTA AGA ACA GGT GAT GAA
781 TTT GCT ACA GGA ACA TTT TTT TTT GAT TGT AAA CCA TGT
820 AGA CTA ACA CAT ACA TGG CAA ACA AAC AGA GCA TTG GGC
859 TTA CCA CCA TTT CTA AAT TCT TTG CCT CAA TCT GAA GGA
898 GCT ACT AAC TTT GGT GAT ATA GGA GTT CAA CAA GAT AAA
937 AGA CGT GGT GTA ACT CAA ATG GGA AAT ACA GAC TAT ATT
976 ACT GAA GCT ACT ATT ATG AGA CCA GCT GAG GTT GGT TAT
1015 AGT GCA CCA TAT TAT TCT TTT GAA GCG TCT ACA CAA GGG
1054 CCA TTT AAA ATA CCT ATT GCA GCA GGA CGG GGG GGA GCG
1093 CAA ACA GAT GAA AAT CAA GCA GCA GAT GGT GAT CCA AGA
1132 TAT GCA TTT GGT AGA CAA CAT GGT CAA AAA ACT ACT ACA
1171 ACA GGA GAA ACA CCT GAG AGA TTT ACA TAT ATA GCA CAT
1210 CAA GAT ACA GGA AGA TAT CCA GCA GGA GAT TGG ATT CAA
1249 AAT ATT AAC TTT AAC CTT CCT GTA ACA AAT GAT AAT GTA
1288 TTG CTA CCA ACA GAT CCA ATT GGA GGT AAA ACA GGA ATC
1327 AAC TAT ACT AAT ATA TTT AAT ACT TAT GGT CCT TTA ACT
1366 GCA TTA AAT AAT GTA CCA CCA GTT TAT CCA AAT GGT CAA
1405 ATT TGG GAT AAA GAA TTT GAT ACT GAC TTA AAA CCA AGA
1444 CTT CAT GTA AAT GCA CCA TTT GTT TGT CAA AAT AAT TGT
1483 CCT GGT CAA TTA TTT GTA AAA GTT GCG CCT AAT TTA ACA
1522 AAT GAA TAT GAT CCT GAT GCA TCT GCT AAT ATG TCA AGA
1561 ATT GTA ACT TAC TCA GAT TTT TGG TGG AAA GGT AAA TTA
1600 GTA TTT AAA GCT AAA CTA AGA GCA TCT CAT ACT TGG AAT
1639 CCA ATT CAA CAA ATG AGT ATT AAT GTA GAT AAC CAA TTT
1678 AAC TAT CTA CCA AAT AAT ATT GGA GCT ATG AAA ATT GTA
1717 TAT GAA AAA TCT CAA CTA GCA CCT AGA AAA TTA TAT

5. A plasmid according to Claim 1 in which the nucleic acid insert comprises at least one additional exogenous nucleic acid element selected from an immunogen-producing portion of the genome of a second disease causing virus of animals.

6. A method for generating antibody production in an animal against parvovirus which comprises exposing naturally occurring antibody producing cells within the animal's body with a protein immunogen having the general sequence:

Met Ser Asp Gly Ala Val Gln Pro Asp Gly Gly Gln Pro Ala Val
Arg Asn Glu Arg Ala Thr Gly Ser Gly Asn Gly Ser Gly Gly Gly
Gly Gly Gly Gly Ser Gly Gly Val Gly Ile Ser Thr Gly Thr Phe
Asn Asn Gln Thr Glu Phe Lys Phe Leu Glu Asn Gly Trp Val Glu
Ile Thr Ala Asn Ser Ser Arg Leu Val His Leu Asn Met Pro Glu
Ser Glu Asn Tyr Lys Arg Val Val Val Asn Asn Met Asp Lys Thr
Ala Val Lys Gly Asn Met Ala Leu Asp Asp Thr His Val Gln Ile
Val Thr Pro Trp Ser Leu Val Asp Ala Asn Ala Trp Gly Val Trp
Phe Asn Pro Gly Asp Trp Gln Leu Ile Val Asn Thr Met Ser Glu
Leu His Leu Val Ser Phe Glu Gln Glu Ile Phe Asn Val Val Leu
Lys Thr Val Ser Glu Ser Ala Thr Gln Pro Pro Thr Lys Val Tyr
Asn Asn Asp Leu Thr Ala Ser Leu Met Val Ala Leu Asp Ser Asn
Asn Thr Met Pro Phe Thr Pro Ala Ala Met Arg Ser Glu Thr Leu
Gly Phe Tyr Pro Trp Lys Pro Thr Ile Pro Thr Pro Trp Arg Tyr
Tyr Phe Gln Trp Asp Arg Thr Leu Ile Pro Ser His Thr Gly Thr
Ser Gly Thr Pro Thr Asn Ile Tyr His Gly Thr Asp Pro Asp Asp
Val Gln Phe Tyr Thr Ile Glu Asn Ser Val Pro Val His Leu Leu
Arg Thr Gly Asp Glu Phe Ala Thr Gly Thr Phe Phe Phe Asp Cys
Lys Pro Cys Arg Leu Thr His Thr Trp Gln Thr Asn Arg Ala Leu
Gly Leu Pro Pro Phe Leu Asn Ser Leu Pro Gln Ser Glu Gly Ala
Thr Asn Phe Gly Asp Ile Gly Val Gln Gln Asp Lys Arg Arg Gly
Val Thr Gln Met Gly Asn Thr Asp Tyr Ile Thr Glu Ala Thr Ile
Met Arg Pro Ala Glu Val Gly Tyr Ser Ala Pro Tyr Tyr Ser Phe
Glu Ala Ser Thr Gln Gly Pro Phe Lys Ile Pro Ile Ala Ala Gly
Arg Gly Gly Ala Gln Thr Asp Glu Asn Gln Ala Ala Asp Gly Asp
Pro Arg Tyr Ala Phe Gly Arg Gln His Gly Gln Lys Thr Thr Thr
Thr Gly Glu Thr Pro Glu Arg Phe Thr Tyr Ile Ala His Gln Asp
Thr Gly Arg Tyr Pro Ala Gly Asp Trp Ile Gln Asn Ile Asn Phe
Asn Leu Pro Val Thr Asn Asp Asn Val Leu Leu Pro Thr Asp Pro

Ile Gly Gly Lys Thr Gly Ile Asn Tyr Thr Asn Ile Phe Asn Thr
Tyr Gly Pro Leu Thr Ala Leu Asn Asn Val Pro Pro Val Tyr Pro
Asn Gly Gln Ile Trp Asp Lys Glu Phe Asp Thr Asp Leu Lys Pro
Arg Leu His Val Asn Ala Pro Phe Val Cys Gln Asn Asn Cys Pro
Gly Gln Leu Phe Val Lys Val Ala Pro Asn Leu Thr Asn Glu Tyr
Asp Pro Asp Ala Ser Ala Asn Met Ser Arg Ile Val Thr Tyr Ser
Asp Phe Trp Trp Lys Gly Lys Leu Val Phe Lys Ala Lys Leu Arg
Ala Ser His Thr Trp Asn Pro Ile Gln Gln Met Ser Ile Asn Val
Asp Asn Gln Phe Asn Tyr Leu Pro Asn Asn Ile Gly Ala Met Lys
Ile Val Tyr Glu Lys Ser Gln Leu Ala Pro Arg Lys Leu Tyr

or substantial equivalents thereof.

7. An infectious animal virus capable of undergoing replication in an animal host and having a heterologous nucleic acid genome containing an exogenous nucleic acid element inserted within the genome and selected from an immunogen-producing portion of the genome of a disease causing virus of animals.

8. A virus according to Claim 7 wherein the genome contains at least one additional exogenous nucleic acid element inserted within the genome and selected from an immunogen-producing portion of the genome of a second disease causing virus of animals.

9. A virus according to Claim 7 wherein the infectious animal virus is from the group of raccoon pox virus, fowl poxvirus, vaccinia, and herpes virus.

10. A virus according to Claim 7 wherein the disease causing virus of animals is from the group of feline parvovirus, canine parvovirus, and feline infectious peritonitis virus.

11. A method for the protection of an animal against a viral disease which comprises:

providing an infectious recombinant animal virus capable of undergoing replication in the animal and having a heterologous nucleic acid genome containing an exogenous nucleic acid element inserted within the genome and selected from an immunogen-producing portion of the genome of the viral disease; and

administering the recombinant animal virus to the animal in an amount sufficient to bring about antibody production against the immunogen produced within the body of the animal.

12. The method according to Claim 11 which comprises administering the recombinant animal virus to the animal by oral, subcutaneous or intranasal routes of administration.

13. An infectious animal virus having a heterologous nucleic acid genome containing an exogenous nucleic acid element inserted within the genome and selected from an immunogen-producing portion of the genome of a disease causing virus of animals, said infectious animal virus having the capability to replicate sufficiently in a host animal to produce sufficient immunogen to bring about an immune response in the animal.

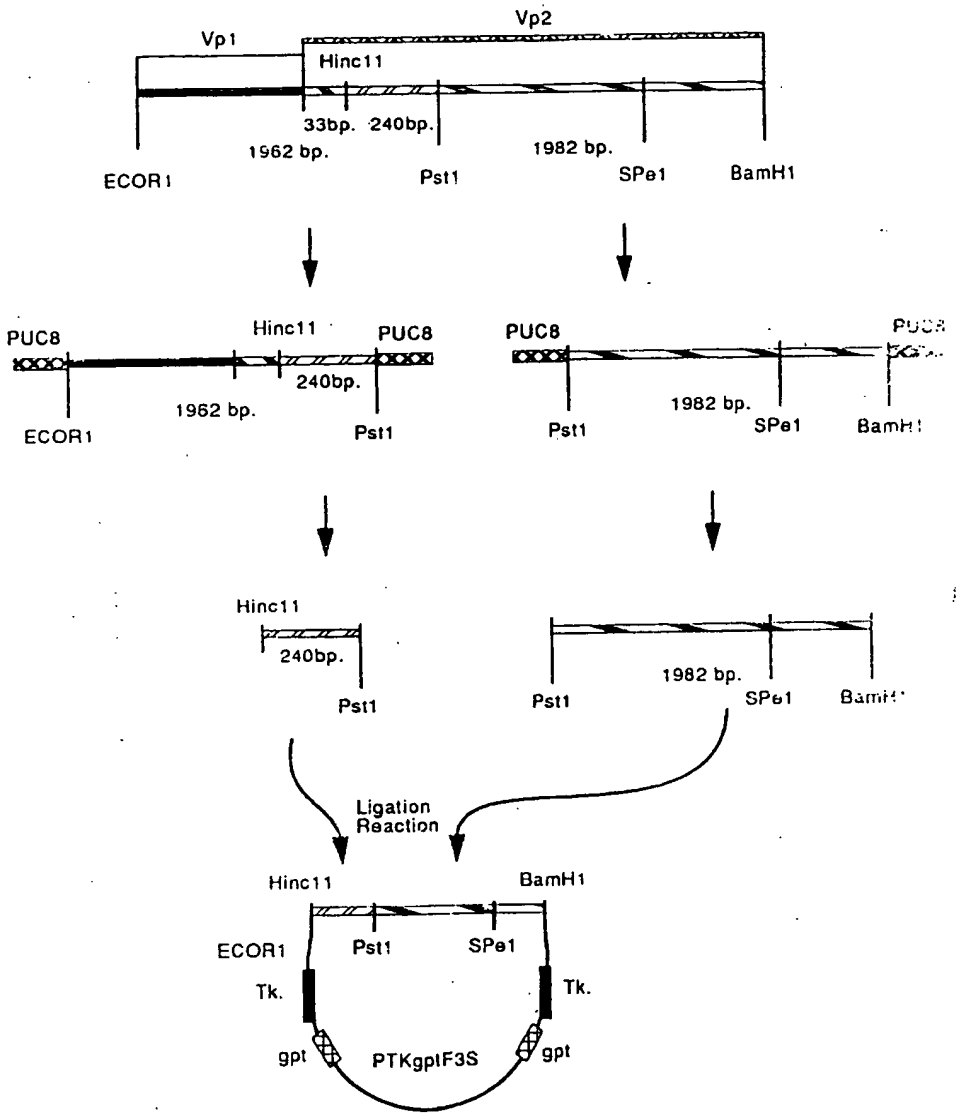
Please refer to content paper # 5 to reference the Oath.

As Originally Filed

FIGURE 1

Subcloning Strategy

Restriction map of complete feline parvovirus DNA.



As Originally Filed

FIGURE 2

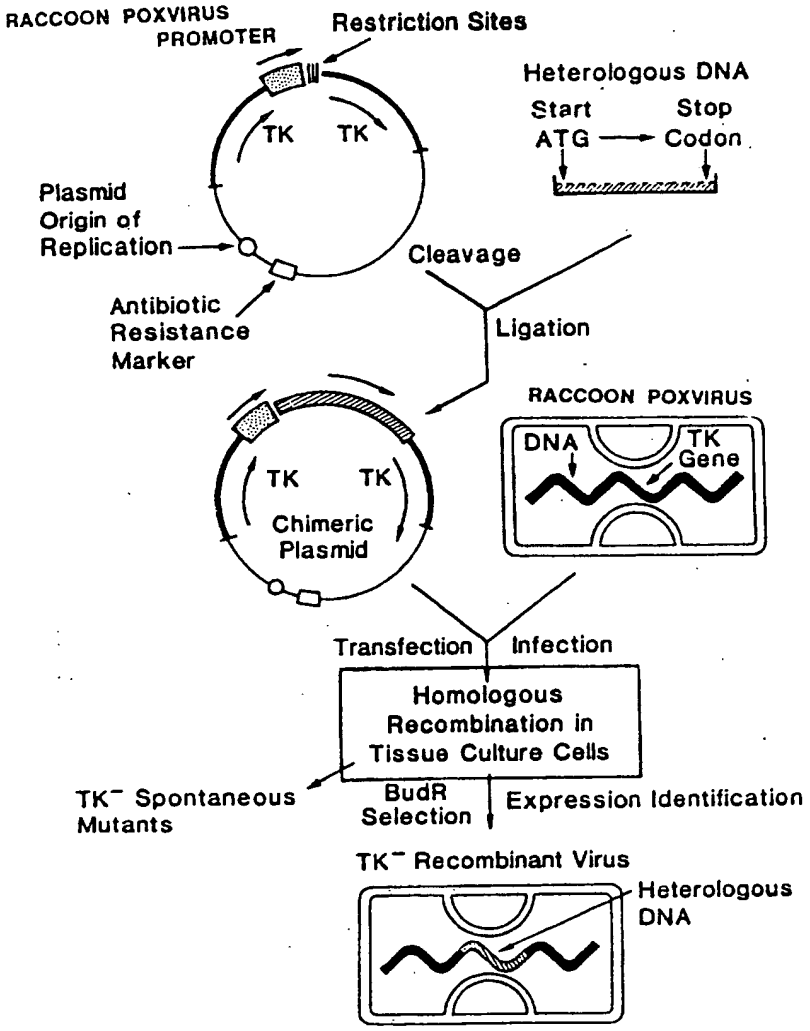


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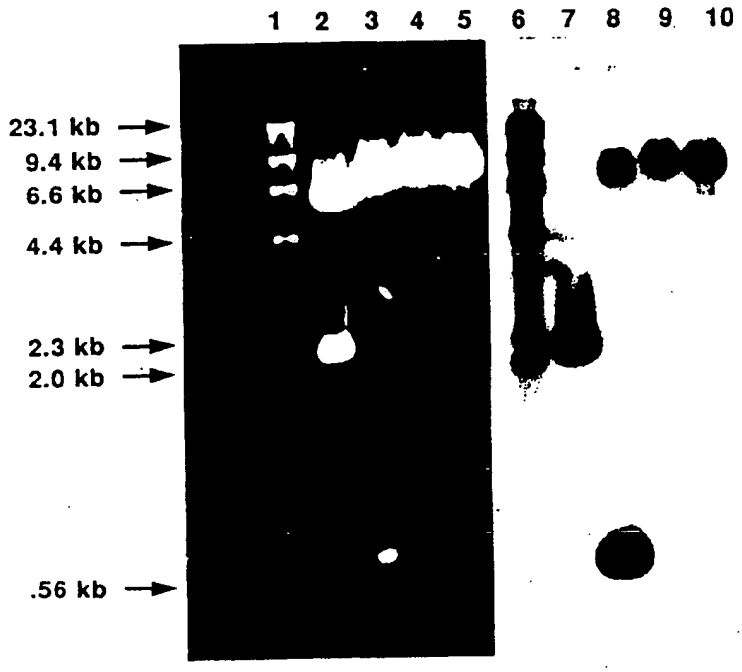
FIGURE 3



As Originally Filed

IL 726609

FIGURE 4

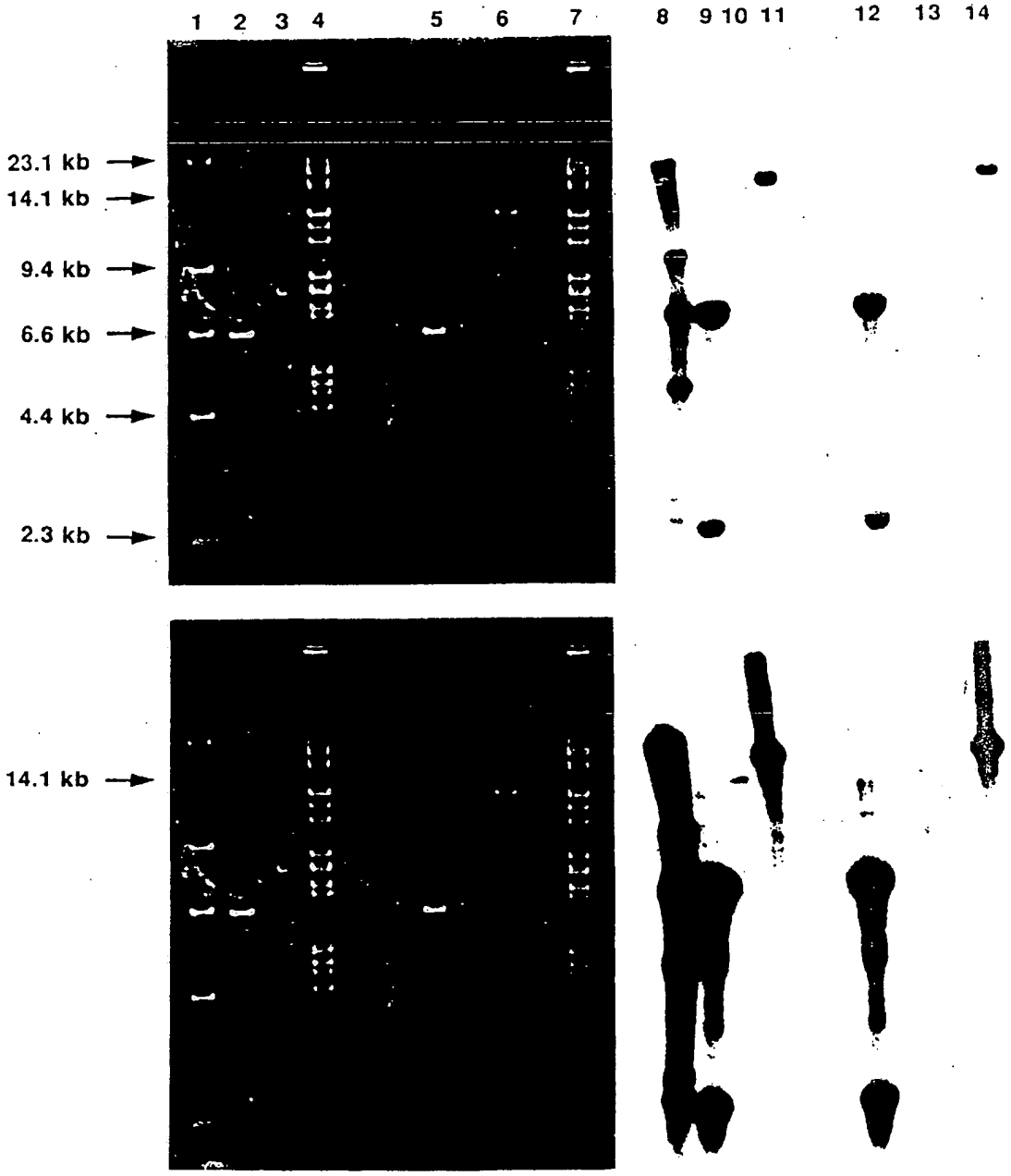


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FIGURE 5

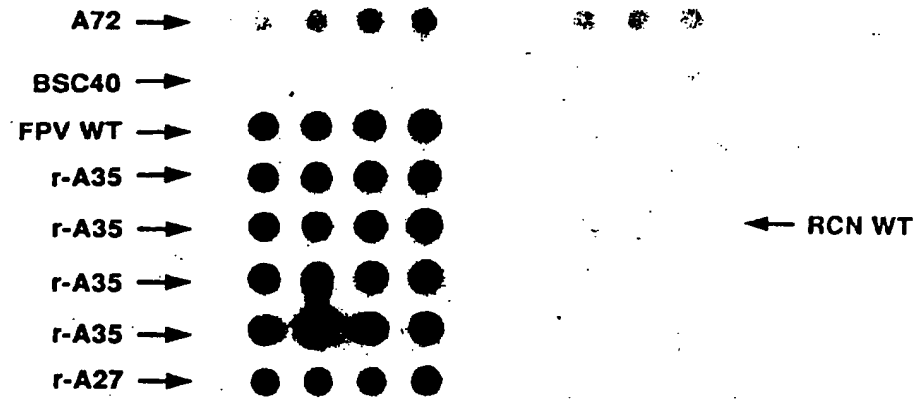

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FIGURE 6A

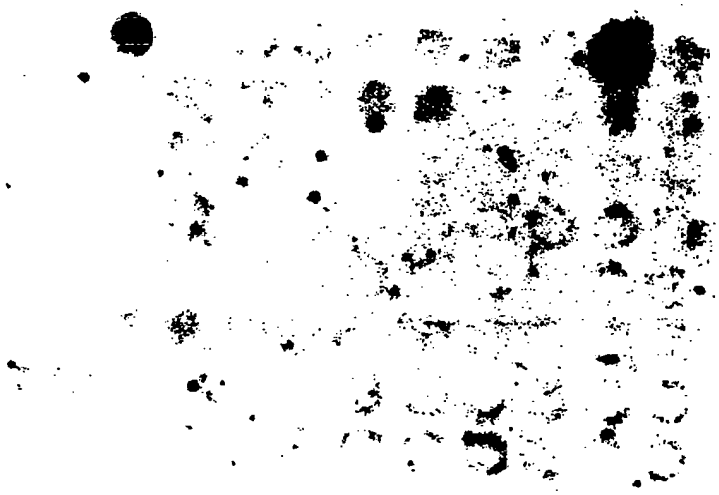

FIGURE 6B